PENICILLIN AND STREPTOMYCIN IN THE TREATMENT OF EXPERIMENTAL ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION OF MICE; WITH OBSERVATIONS ON IMMUNOLOGIC REACTION TO THE INFECTION*

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In a previous communication (1) we reported the results of determinations of the therapeutic effect of sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine in mice inoculated with *Erysipelothrix rhusiopathiae*. In this study 12.5 per cent of mice treated before or after the administration of these compounds survived. The therapeutic effect of these compounds is therefore limited. A report was also made of the ineffective use of the sulfonamide compounds in treatment of patients with erysipeloid of Rosenbach and in treatment of one patient with the septicemic form of the infection (2).

The purpose of the present investigation was to determine the therapeutic effects of penicillin and streptomycin in the treatment of mice inoculated with *Erysipelothrix rhusiopathiae*.

METHOD OF STUDY

A pure culture of *Erysipelothrix rhusiopathiae* maintained in lyophilic form was employed. The organism was originally obtained from swine, the site of "diamond" skin disease (a mild form of swine erysipelas (3)). Morphology and virulence have remained unchanged. Sterile water was added to the lyophilic culture and injected intraperitoneally into white mice. The heart blood of moribund animals were cultured in heart infusion broth. It was observed that 0.2 cc. of a 1:10,000 dilution of a forty-eight hour culture killed mice in three to ten days. This amount injected intraperitoneally was used in the three experiments shown in Table 1. In order to maintain virulence, heart blood of mice dying of the infection was cultured and a forty-eight hour culture was employed for each set of mice in each experiment. This procedure increased the virulence of the organism. In the experiments shown in Tables 2 and 3, 0.2 cc of a 1:25,000 dilution was therefore employed.

A total of 66 Swiss mice, ranging in weight from 19 to 22 gms. were used in the penicillin study and 52 in the streptomycin study. Mice used in the control group were inoculated with the same culture in the same dose as was employed for the treated animals.

The sodium salt of penicillin was used in sterile water and was injected subcutaneously at 9 A.M., 12 Noon, 3 P.M., 6 P.M., and 9 P.M. The number of units at 9 P.M. was twice the number given for the four preceding doses and was administered in peanut oil-beeswax. Treatment was given either for four or five days in succession. Different groups of mice were treated with varying amounts of penicillin either immediately after inoculation, eight hours after, and sixteen hours after. Streptomycin was given in varying number of units in the same manner and at the same intervals as that employed for penicillin. Treatment was given either immediately after inoculation or eight hours after and was continued for five consecutive days.

Results. The three experiments shown in Table 1 involved 26 mice treated with penicillin and 14 control mice. Treatment ranged from 20 units at each

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injection or 120 units daily for four days to 200 units at each injection or 1200 units daily. Of the twenty-six treated mice, twenty-three died. Of two mice that survived, one received 720 units daily for four days, the other 1200 units daily. This treatment was started 16 hours after inoculation. The remaining animal that survived was one of three that received 1200 units daily for four days. This treatment was given immediately after inoculation. All of the untreated mice died from the second to the tenth day after inoculation.

The two experiments shown in Table 2 involved 20 mice treated with penicillin and 8 control mice. Two of three mice survived that were treated with

TABLE 1

Therapeutic effect of penicillin in treatment of mice inoculated with Ery. rhusiopathiae[1]

TREATMENT	UNITS PER MOUSE[2]	NO. OF MICE	DEATHS												
			In days												Total
			1	2	3	4	5	6	7	8	9	10	12	14	
16 hours after inoculation	20	2						1	1						2
	40	2					1					1			2
	120	2			1										1
	200	2								1					1
Control		6			1	1	1		1		1	1			6
8 hours after inoculation	40	3					2	1							3
	120	3					1	1	1						3
	200	3						1	1	1					3
Immediately after inoculation	40	3					1		2						3
	120	3					1	1		1					3
	200	3						2	2						2
Control		6		5	1										6

[1] By intraperitoneal injection of 0.2 cc. of 1:100,000 dilution of a 48 hour in heart infusion broth.

[2] Subcutaneously every 3 hrs., 5 doses daily for 4 consecutive days. The dose of last injection on each day was double that of the preceding four and was given in peanut oil-beeswax.

600 units daily for five days, and two of three mice survived that were treated with 1200 units daily for five days. This treatment was given eight hours after inoculation. All of four mice treated with 1800 units daily given eight hours after inoculation survived. Of the ten mice treated with either 600, 1200 and 1800 units daily for five days given immediately after inoculation all survived. All of the untreated mice died from the second to the sixth day after inoculation.

The experiments shown in Table 3 involved 48 mice treated with streptomycin and 4 control mice. Treatment ranged from 20 units at each injection or 120 units daily for five days to 600 units at each injection or 3600 units daily for five days. Of the 48 treated mice, forty-four died. Of the surviving mice, two

were of a group of four treated with 3600 units daily administered eight hours after inoculation. Of the remaining two surviving mice, one was of a group of four treated with 240 units daily, the other was of a group of four treated with 1200 units daily. All of the untreated mice died from the fourth to the eight day after inoculation.

TABLE 2

Therapeutic effect of penicillin in treatment of mice inoculated with Ery. Rhusiopathiae[1]

TREATMENT	UNITS PER MOUSE[2]	NO. OF MICE	DEATHS											
			In days											Total
			2	3	4	5	6	7	8	9	10	14	21	
8 hours after inoculation	100	3								1	1			2
	200	3						1	1					2
	300	4												
Immediately after inoculation	100	3												
	200	3												
	300	4												
Control.....		8	1	1	2	3	1							8

[1] By intraperit. injec. 0.2 cc. of 1:25,000 dil. of a 48 hour culture in heart infusion broth.

[2] Subcutaneously every 3 hours, 5 doses daily for 5 consecutive days. The dose of the last injection on each day was double that of the preceding four and was given in peanut oil-beeswax.

COMMENT

Penicillin was effective in treatment of mice with septicemic form of *Erysipelothrix rhusiopathiae*. The survival rate was 100 per cent following treatment with 600 units daily (the smallest amount employed) administered immediately after inoculation and after treatment with 1800 units daily administered eight hours after inoculation. The survival rate was 50 per cent after treatment with either 600 or 1200 units daily administered eight hours after inoculation.

Calculating on the basis of 100 units per injection (600 units daily), the corresponding dose for man (with septicemic infection) would be a total of about 2,100,000 units administered daily in five injections for five days.

Streptomycin, on the other hand, showed very slight therapeutic effect. Only 4 mice survived of 48 treated with varying number of units up to 3600 units daily. Some evidence of therapeutic effect was the longer period the animals lived that received the higher number of units in comparison with the control animals.

It is not possible to produce in mice a local or cutaneous form of infection corresponding to erysipeloid of Rosenbach. It was therefore not possible to experimentally study the value of penicillin administered locally or by parenteral injection in treatment of erysipeloid.

Heilman and Herrell (4) reported in vitro and in vivo studies of the effect of penicillin on *Erysipelothrix rhusiopathiae*. Growth of the organism was inhibited in the tubes containing

0.1 of a unit of penicillin per cubic centimeter and partially inhibited in the tubes containing 0.01 unit per cubic centimeter. In vivo studies embraced experiments in which the dilution of the bacterial inoculums varied and treatment with penicillin was started immediately after inoculation, 16 hours and 21 hours after. Penicillin was administered subcutaneously at the rate of 1,000 units per day in divided doses. Both the sodium and the calcium salts of penicillin were used. The mice received 125 units dissolved in physiologic salt solution at 9 A.M., 12 Noon, 3 P.M., and 6 P.M. and 500 units of ground penicillin suspended in sesame oil at 9 P.M. Treatment continued for four days in some experiments and seven days in other experiments. Of forty treated mice, two died, a mortality rate of 5 per cent. The two treated mice that died did so on the fourth and seventh days after treatment was stopped. They were from the experiment in which treatment was not started until twenty-one hours after inoculation and was continued for only four days.

Harvey, Libby and Waller (5) studied the effect of penicillin administered orally to mice inoculated with lethal dose of *Erysipelothrix rhusiopathiae*. Penicillin administered in feed or in drinking water afforded 100 per cent protection provided its use was instituted within twenty-four hours after inoculation. Much larger amounts of penicillin was required than by parenteral injections. One thousand units of penicillin per 1 cc. of drinking water was protective, 500 units per 1 cc. afforded 20 per cent protection, and amounts lower than 500 units gave no protection. Fifty cubic centimeters of water containing 1000 units of penicillin was consumed by 5 mice in 48 hours. The effective average intake per mouse was 5000 units per cc. The length of continuous treatment varied from 24 hours to 10 days. Treatment started 48 hours after inoculation was ineffective even in doses as high as 10,000 units per one cc. of water.

Van Es, Olney and Blore (6) studied the effect of penicillin in experimentally produced infection in pigeons.[1] Their experiments were planned to furnish basis for penicillin treatment of the infection in swine. It was observed that 1200 units of penicillin per gram of net weight (minus weight of feathers) when injected at time of inoculation with lethal dose of *Erysipelothrix rhusiopathiae* failed to protect. Protection began when 2400 units was employed. If treatment was delayed even less than 24 hours after inoculation, no protection was afforded. Pigeons that survived after penicillin treatment were re-inoculated with lethal dose of *Erysipelothrix rhusiopathiae* to determine if they were immune. Immunity did not result excepting in the pigeons receiving initial treatment with penicillin 24 or 48 hours after virulent inoculation. Best results were obtained with repeated injections of penicillin. The authors concluded that such procedure would consitute limitation in the use of penicillin treatment of the infection in swine.

IMMUNOLOGIC REACTION TO ERYSIPELOTHRIX RHUSIOPATHIAE

It was observed in this study that mice surviving after penicillin treatment were not killed after re-inoculation with eight times the lethal dose of *Erysipelothrix rhusiopathiae*. Agglutination tests performed with the serum of some of the mice surviving the re-inoculation were negative in titre of 1–25.[2]

The observation that immunity ensued in mice and pigeons treated with penicillin provided there was a delay in starting penicillin treatment after inoculation is consistent with the following observation. As shown in Table 3, two of four mice survived that were treated eight hours after inoculation with 3600 units daily of streptomycin (the largest amount employed), whereas of four mice treated with the same amount immediately after the inoculation succumbed.

[1] It is of interest to note that pigeons were selected in their study since swine, though susceptible to naturally occurring infection, cannot readily be infected experimentally.

[2] Agglutination tests were performed by Dr. George W. Stiles, U. S. Department of Agriculture, Bureau of Animal Industry.

This suggested increasing the dose of streptomycin and delaying treatment beyond eight hours after inoculation. This experiment was not performed since penicillin was effective in treatment.

A reasonable explanation of immunity ensuing after delay in starting penicillin treatment is the opportunity for consummation of immunologic reaction of the host of the infection.[3]

TABLE 3

Therapeutic effect of streptomycin in treatment of mice inoculated with Ery. rhusiopathiae[1]

TREATMENT	UNITS PER MOUSE[2]	NO. OF MICE	DEATHS In days												Total
			1	2	3	4	5	6	7	8	9	10	12	14	
8 hours after inoculation	20	4					2	2							4
	40	4					2	2							4
	100	4					2	1	1						4
	200	4					2	2							4
	400	4				1	1	2							4
	600	4										1	1		2
Immediately after inoculation	20	4				1	1	1	1						4
	40	4					1	1	1						3
	100	4					2	1	1						4
	200	4						1	1	1					3
	400	4									2	1	1		4
	600	4								1		1	2		4
Control.....		4				2	1			1					4

[1] By intraperitoneal injection of 0.2 cc. of 1:10,000 dilution of a 48 hour culture in heart infusion broth.

[2] Subcutaneously every 3 hours, 5 doses daily for 5 consecutive days. The dose of the last injection on each day was double that of the preceding four and was given in peanut oil-beeswax.

The development of immunity of mice and pigeons is consistent with the experimental immunity of horses in the development of an antiserum and the demonstration that rabbits can also be immunized.

Apparently immunity is not entirely dependent upon the formation of agglutinins since agglutination tests conducted with the serums of immune mice were negative. Agglutination tests with the serum of infected swine give in-

[3] A counterpart of this phenomenon is the occurrence of neurorecidive or iridorecidive in patients *inadequately* treated in the *early* stage of syphilis. A few injections of a spirocheticidal medicament followed by cessation of treatment interferes with the consummation of immunologic reaction to the infection. After a certain period in early syphilis, inadequate treatment is not followed by neurorecidive or iridorecidive. In the history of syphilis some clinicians purposely delayed treatment until the appearance of the secondary eruption. The dictum is recalled that patients with a florid secondary eruption was less likely to have neurosyphilis.

constant results. Grey, Osteen and Schoening (7) studied the value of the agglutination test in the diagnosis of the infection in swine. In the acute stage they observed that, although the test may be positive, this was quite variable. More uniform results were obtained in animals with joint lesions visible on clinical examination. Karlson and McNutt (8) observed that microscopic agglutination tests conducted on swine dead or dying of the septicemic form of the disease gave negative results.

Agglutination tests with the serum of patients with erysipeloid in my experience have yielded inconstant results and are unreliable as a diagnostic aid.[4] This is in agreement with the studies of Bierbaum and Gottron (9) who observed agglutination did not exceed dilutions of serum ranging from 1:20 to 1:40 in which dilutions the serums of normal persons caused agglutination.

Local infection (erysipeloid) does not produce immunity since second attacks of the infection occur. Blood transfusions from several donors who had had erysipeloid were ineffective in treatment of the patient the senior author reported (10) who had generalized cutaneous form of infection with arthritic and constitutional symptoms.

Fortunately septicemic infection in man is rare, little opportunity has been presented to study immunologic reaction to this form of infection. From the aforegoing considerations recovery from septicemic infection would convey immunity.

SUMMARY

Penicillin and streptomycin were employed in treatment of experimentally produced septicemic form of Erysipelothrix rhusiopathiae infection in mice. Treatment was administered in 5 daily injections for five successive days. The last of the daily injections was twice the amount of the preceding four.

Following penicillin treatment the survival rate was 100 per cent with 600 units daily administered immediately after inoculation; and after treatment with 1,800 units daily beginning 8 hours after inoculation. The survival rate was 50 per cent after treatment with either 600 or 1,200 units daily administered 8 hours after inoculation.

Streptomycin showed very slight therapeutic effect. Only four mice survived of 48 treated with varying number of units up to 3,600 units daily.

Immunity ensued in mice that survived penicillin treatment. Such animals were not killed when re-inoculated with eight times the lethal dose of Erysipelothrix rhusiopathiae.

The immunologic reaction of man and animals to Erysipelothrix rhusiopathiae infection is discussed.

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